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Dual Mechanisms of Twitching During Sleep in Neonatal Rats

Mark S. Blumberg and Dennis E. Lucas

Twitches of the limbs during REM sleep in adult mammals result from descending motor activation from the brainstem. In contrast, many spontaneous movements in embryos appear similar to REM-related twitches and result from the local firing of spinal motor neurons. To determine which mechanism produces twitches in neonates, we analyzed twitching in 5- and 8-day-old rat pups that had spinal cords transected in the lower thoracic region. This transection separated motor units controlling forelimb movements from motor units controlling hindlimb movements. Spinal transection did not significantly affect the amount of forelimb twitching. In contrast, the amount of hindlimb twitching in transected pups was reduced by only 35%–50%. Given that hindlimb twitching was not eliminated by spinal transection, it is concluded that there are 2 independent mechanisms producing twitches at these ages.

The movements of animals during sleep and the possible connection of these movements to the activity of dreaming have fascinated scientists for years. Darwin took the "movements and voice" of animals during sleep as an indication of dreaming and, thus, "some power of imagination" (1871/1981, p. 46). Similarly, Darwin's student, Romanes, suspected "that ferrets dream, as I have frequently seen them when fast asleep moving their noses and twitching their claws as if in pursuit of rabbits" (1883/1977, p. 347). Today, we understand that such twitches are but one indicator of a stage of sleep called REM sleep, identified in adult mammals by the presence of a number of behavioral and electrophysiological components. These components include a desynchronized EEG, muscle atonia, phasic muscular twitches, and rapid eye movements (Vertes, 1984); they are easily measured in adult mammals, and, moreover, they provide the clear impression of a distinct behavioral state in adults. In contrast, altricial neonates do not exhibit a distinct REM state as judged by these adult criteria; their EEG is less cohesive, their postural control is minimal, and rapid eye movements are rare. These factors have forced a reliance on twitching as a marker for REM sleep in neonates. In fact, altricial neonates twitch 70%–80% of the time, suggesting that REM sleep is their predominant behavioral state (Gramsbergen, Schwartze, & Prechtl, 1970; Jouvet-Mounier, Astic, & Lacote, 1970).

The functional significance of twitching is not easy to assess. Sleep researchers, whose experimental subjects are primarily adults, consider twitching to be generated by motor neurons within the brainstem whose activity "leaks through" the REM-related inhibition of muscle tone (Chase & Morales, 1990; Vertes, 1984). On the other hand, neuroembryologists consider twitching a ubiquitous component of early vertebrate development generated by local circuits within the spinal cord, not by descending influences from the brainstem (e.g., Hamburger's Type I movements; Hamburger, 1973; Narayanan, Fox, & Hamburger, 1971).

Such a disjunction between the reality of REM sleep in neonates and its current conceptualization by sleep researchers suggests that increased understanding of the control, coordination, and development of myoclonic twitching in neonates could provide valuable insight into the function of REM sleep. In the present study, we integrated the two conceptions of twitching by investigating the mechanisms underlying twitching in rats at 5 and 8 days of age, a time at which descending spinal influences are only beginning to mature (Stelzner, 1985). By removing descending control of the hindlimbs through thoracic spinal transection and by analyzing the temporal distribution of twitching above and below the transection, we had evidence for the simultaneous presence of both local and descending activation of twitching movements in neonates.

Method

Experimental subjects. Twenty-four pups (12 males and 12 females) from six litters were used. All pups were born to Harlan Sprague-Dawley females in the animal colony at the University of Iowa. The pups were raised in litters that were culled to 8 pups within 2 days after birth (day of birth = Day 0). Litters and mothers were housed in standard laboratory cages (48 × 20 × 26 cm) in which food and water were available ad libitum. All rats were maintained on a 12-hr light–dark cycle with lights on at 6 a.m.

Surgery. All surgeries were performed when pups were 1 to 2 days of age. On the day of surgery, test pups were weighed, their sex was determined, and identification marks were applied. Four pups in each litter were assigned to one of three experimental groups: 2 pups were assigned to the transected group, and 1 pup each was assigned to the sham-transected and non-surgical control groups. Two pups were assigned to the transection group in order to increase the likelihood that at least 1 pup in each of the six litters received a complete transection.

Spinal transections were performed as described in Stelzner, Ershler, and Weber (1975). Pups were anesthetized by immersion in

Mark S. Blumberg, Department of Psychology, University of Iowa; Dennis E. Lucas, Neuroscience Program, University of Iowa.

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Correspondence concerning this article should be addressed to Mark S. Blumberg, Department of Psychology, University of Iowa, Iowa City, Iowa 52242. Electronic mail may be sent to mark-blumberg@uiowa.edu.

ice water for at least 4 min, by which time they no longer exhibited reflexive responses to tactile stimulation (Phifer & Terry, 1986). After removal from the ice water, they were placed on a pack of blue ice for the duration of the surgery. Using clean surgical technique and a stereomicroscope, we made a midline dorsal incision that extended from the brown fat pad caudal to the level of the lumbar spinal cord. Tissue overlying the spinal cord was cleared away, and the vertebral column was visualized. In the mid-to-lower thoracic region, parts of the dorsal column were removed, and the spinal cord was visualized. Then, using a fine angled needle, we cut the cord; special care was taken to scrape around the inside edges of the vertebral column to ensure complete transection. After transection was complete and bleeding controlled, the pup's skin was sutured closed. After surgery, pups were placed in an infant incubator set at a temperature of 35–36 °C. After a few hours when pups had recovered from the anesthesia, they were returned to their home cage. At this time, there were never more than 8 pups in the litter to ensure that the recovering pups had easy access to maternal milk. The sham control pups experienced the identical surgical procedure—through visualization of the vertebral column—but their cords were not cut.

Behavioral recording. At 5 and 8 days of age, transected, sham, and nonsurgical pups were removed from their home cage, weighed, and placed in an infant incubator. The temperature and relative humidity within the incubator were maintained between 34.5–35.8 °C and 50%–64%, respectively. The air temperature was within the thermoneutral zone of pups at the ages tested (Spiers & Adair, 1986). At the time of testing, all pups had been fed recently, as evidenced by the presence of milk clearly visible through their abdominal skin.

Previous researchers (e.g., Jouvet-Mounier et al., 1970; Gramsbergen et al., 1970) have quantified twitch behaviors in unrestrained rats. For our purposes, however, we needed to situate our rats in such a way that all of their limbs were clearly observable. Moreover, because we wished to compare fine limb movements, we also needed to minimize disruptions caused by postural shifts that obscure movements in one or more limbs and cause interference and resistance from the substrate. In preliminary experiments, we placed pups on their backs on a felt surface with elastic strips very loosely fastened over the ventrum to maintain this supine posture. (We found that very little contact between the elastic strips and the pups' bodies was sufficient to prevent the pups from rolling over onto their abdomens.) Pups quickly adapted to this situation as evidenced by the fact that, within minutes, pups began exhibiting the myoclonic twitches indicative of REM sleep. As our results indicated, pups exhibited very high levels of twitching throughout the 10-min test period. Indeed, we observed very few instances of behavioral agitation (e.g., kicking, twisting, and pushing) in response to the mild restraint.

Video recording began 35–120 min after pups had been placed in the incubator; thus, all pups were thermally stable when recording began. Recording continued for 10 min, after which time the pups were returned to their home cages.

Behavioral confirmation of transections. When pups were 16 days of age, video recordings were made of each pup's ability to walk, rear, and climb. To examine walking, we placed a pup on a padded surface and allowed it to roam freely. To examine rearing, we placed a pup on the same padded surface but inside a Plexiglas cylinder (diameter: 12 cm; height: 10 cm). Pilot observations indicated that normal pups regularly reared under these conditions and touched the upper part of the walls of the cylinder with their forepaws. Climbing was examined by draping the forelimbs of pups over the top of the same Plexiglas cylinder; again, pilot observations indicated that normal pups used their hindlimbs to climb up and over the cylinder wall. In addition, audible vocalizations in response to tail pinching were used as one measure of the completeness of the lesion.

Histological confirmation of transections. After the behavioral testing when pups were 16 days of age, all subjects were overdosed with sodium pentobarbitol and perfused through the heart with saline and formalin. The spinal column was removed from each pup and placed in formalin for fixation.

Data collection. To score the videotaped data, we wrote an event-recorder program on HyperCard for the Macintosh. When a designated key on the keyboard was pressed, the computer recorded the key pressed and the time at which it was pressed. Data collection was performed simultaneously by two observers, each of whom was responsible for scoring twitching either in the forelimbs or hindlimbs (including the tail; hereafter, all references to the hindlimbs include the tail). These observer assignments to the forelimb and hindlimb groups were counterbalanced across the six litters.

The tail was included in the scoring of the hindlimbs for the following reasons: First, pilot observations indicated a high correlation between tail twitches and hindlimb twitches. Second, it was apparent that movements of the tail sometimes resulted in passive hindlimb movements, thus making it difficult to reliably distinguish between tail and hindlimb twitching. And third, any hypotheses regarding the mechanisms underlying hindlimb twitching would apply equally well to tail twitching. Thus, including the tail in the scoring process made determination of hindlimb twitching easier without a significant loss of information.

A twitch was defined as a phasic, rapid, independent, and uncontrolled movement of any part of the focal limbs (Gramsbergen et al., 1970). Observers were careful to distinguish an active movement of the limb from a passive movement that may have resulted from activity in other parts of the body. Simultaneous twitching movements of, for example, the left and right forelimbs were scored as a single twitch. Limb movements indicative of an awake rat (e.g., stepping and stretching) were easily distinguishable from twitching movements. Twitches sometimes occurred in close temporal proximity to "startles" (characterized by the simultaneous contraction of many body muscles; Gramsbergen et al., 1970); these twitches were counted as such if they appeared independent of movements in other parts of the body. Finally, in pilot observations, two experienced, independent observers unaware of the conditions of the pups were able to distinguish twitching from nontwitching movements with high interrater reliability (median exact probability = 90%, $n = 72$).

Data analysis. Temporal data were stored on computer disk until analyzed. These data were imported to Statview 4.0 for the Macintosh for statistical analysis. Differences between groups were tested with analysis of variance (ANOVA); unless otherwise indicated, the post hoc test was Fisher's protected least significant difference and the significance level was $p < .05$. Finally, the measures of interlimb synchrony are expressed as proportions: These scores were arcsine root transformed before statistical analysis to meet the requirements of normative statistics (arcsine $\sqrt{P}$ where P is the proportion; Pollard, 1977).

Results

Selection of transected pups for analysis. As described above, 2 pups from each litter underwent spinal transection to increase the likelihood that at least 1 pup with a completely transected spinal cord survived. In one case, a transected pup died 2 days after surgery. To select from the remaining five pairs of transected pups, we used behavioral observations.

First, the tails of all 16-day-old pups were pinched to elicit a vocalization. This is usually the hardest response to eradicate after spinal transection because the sensory fibers for this response traverse laterally in the spinal cord. All sham and nonsurgical pups emitted clear and reliable vocalizations in response to tail pinching. In contrast, all but 1 transected pup

were silent; the one exception vocalized intermittently in response to tail pinching and was excluded from data analysis.

When the motor behaviors of transected pups were examined at 16 days of age, the rats exhibited clear behavioral deficits in relation to the sham and nonsurgical controls. The transected pups could not use their hindlimbs while walking. There was, however, some postural control of the hindlimbs as well as some retention of the placing response, as described by Stelzner et al. (1975). When placed in the middle of a small Plexiglas cylinder, sham and nonsurgical pups exhibited many rearing responses up the wall of the cylinder, whereas the transected pups never reared in this situation. Finally, when the pups' forelimbs were draped over the top of the cylinder and the pups were hanging from this position, sham and nonsurgical pups were able to use their hindlimbs to climb up and over the cylinder, whereas the transected pups were never able to do this.

Relative performance on these tests was used to select among the remaining four pairs of transected pups. In each case, the rat exhibiting the most extensive behavioral deficits was chosen for analysis. For example, 1 pup exhibited some retention of motor function on one side of the body and was excluded from the analysis; when its spinal cord was examined later, it was found that the transection was incomplete.

When the spinal cords of the 6 transected pups selected for data analysis were examined visually under a stereomicroscope, all exhibited complete transections. It was evident that the meninges had closed over the surgical wound and that CSF had filled the gap between the anterior and posterior sections of the spinal cord. In addition, 1 transected pup's spinal cord was sectioned, stained, and examined microscopically: This procedure verified what was apparent from visual inspection. All transections ranged between T9 and T11, as determined by counting the thoracic segments anterior to the 13th rib.

Body weight. A repeated measures ANOVA was used to test differences in body weights between the three experimental groups on the day of surgery and the 3 postsurgical test days. There were significant main effects of group, $F(2, 15) = 13.6, p < .0005$, and age, $F(3, 45) = 1307.4, p < .0001$, and there was also a significant Group × Age interaction, $F(6, 45) = 41.7, p < .0001$. The source of these effects was examined further with a series of paired t tests comparing the means across groups for each of the 4 days. Pairs were composed of siblings assigned to different experimental groups. Based on the findings of others (Stelzner et al., 1975), it was expected that transected pups would weigh less than the sham and nonsurgical pups on each of the postsurgical days.

On the day of surgery, there were no significant differences in the body weights of pups in the three groups; transected vs. sham: paired $t(5) = .742$, *ns;* transected vs. nonsurgical: paired $t(5) = .867$, *ns;* sham vs. nonsurgical: paired $t(5) = .322$, *ns.* Moreover, at all postsurgical ages tested, the weights of sham and nonsurgical control pups did not differ significantly from each other. In contrast, transected neonates weighed significantly less than controls at all postsurgical ages tested. When 5 days old, transected pups weighed 10.1 ± 0.5 g as compared with 11.8 ± 0.4 g and 12.5 ± 0.3 g for sham and nonsurgical pups, respectively; transected vs. sham: paired $t(5) = 2.90, p < .05$; transected vs. nonsurgical: paired $t(5) = 7.07, p < .001$. When 8 days old, transected pups weighed 15.0 ± 0.8 g as compared with 18.6 ± 0.6 g and 19.8 ± 0.7 g for sham and nonsurgical pups, respectively; transected vs. sham: paired $t(5) = 3.26, p < .05$; transected vs. nonsurgical: paired $t(5) = 9.14, p < .0005$. Finally, the respective values for 16-day-old pups were 29.7 ± 1.3 g, 39.2 ± 1.6 g, and 40.9 ± 1.4 g; transected vs. sham: paired $t(5) = 5.92, p < .005$; transected vs. nonsurgical: paired $t(5) = 14.49, p < .0001$. Other than this weight loss, transected pups appeared healthy.

Effect of spinal transection on forelimb and hindlimb twitching. Behavioral data were analyzed for all pups at both 5 and 8 days of age. Because the results were fundamentally identical at both ages, only the data for the pups at 5 days of age are presented here.

The total number of forelimb twitches was not affected by spinal transection (see Figure 1; $F(2, 17) = 0.08$, *ns*). In contrast, the number of hindlimb twitches in the transected pups was reduced by approximately 50% in relation to the number of hindlimb twitches in the sham and nonsurgical pups (see Figure 1). This reduction was highly significant, $F(2, 17) = 45.52, p < .0001$.

The hindlimb twitching that survived spinal transection appeared to be qualitatively identical to that observed in normal pups, as would be expected from similar experiments in other species demonstrating local spinal sources for embryonic motility (Provine, 1986). Nonetheless, a 50% reduction in hindlimb twitching requires explanation. We discounted general debilitation caused by surgery as an explanation because, as shown above, forelimb twitching was not affected by the surgery.

To analyze further this reduction in hindlimb twitching, the temporal distributions of twitching in the transected, sham, and nonsurgical pups were compared. First, for each twitch within the forelimb or hindlimb groups, we calculated the time since any limb within that same limb group twitched previously (designated the intertwitch interval). Next, the frequency distribution of intertwitch intervals (i.e., between 0 and 1 s, 1

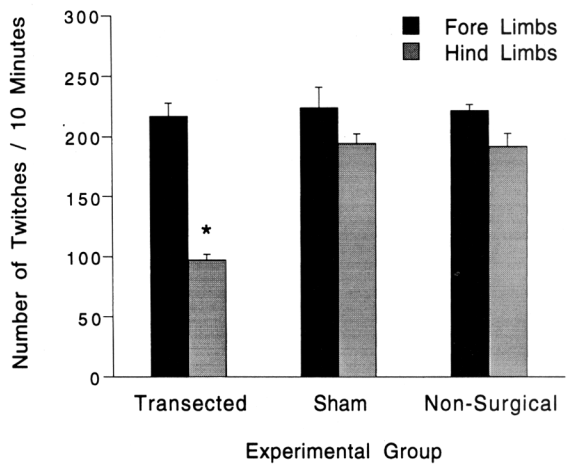

Figure 1. Number of twitches in the forelimbs and hindlimbs during a 10-min observation period for the transected, sham, and nonsurgical pups at 5 days of age. $n = 6$ for each group. Mean ± SE_M. *$p < .0001$ in relation to sham and nonsurgical controls.

and 2 s, etc.) was determined for each pup. Figure 2 presents the frequency distributions for the first 10 intertwitch intervals for the forelimbs (top) and hindlimbs (bottom). For the forelimbs, ANOVA indicated no significant differences between transected, sham, and nonsurgical pups for any of the first 10 intertwitch intervals: $0.084 \leq F(2, 17)\text{'s} \leq 1.94$. For the hindlimbs, however, transected pups exhibited significantly fewer intertwitch intervals in relation to both control groups for intertwitch intervals between 0 and 1 s, $F(2, 17) = 16.54$, $p < .0005$, between 1 and 2 s, $F(2, 17) = 41.58, p < .0001$, and between 2 and 3 s, $F(2, 17) = 11.32, p < .001$. Frequencies of the longer intertwitch intervals were not significantly different; $0.16 \leq F(2, 17)\text{'s} \leq 2.50$. Thus, spinal transection had a specific impact on the expression of hindlimb twitching: short interval twitching was decreased, whereas long interval twitching was unaffected.

Log-survivor plots, such as those in Figure 3, are helpful for illustrating the temporal patterning of events, especially randomly occurring events (Fagen & Young, 1978). The intervals between random events form a Poisson distribution which, on a log-survivor plot, fall along a straight line with a slope that is proportional to the rate at which events occur. The dark squares in Figure 3 depict the temporal distribution of hindlimb twitching in transected pups: The linearity of this distribution, as indicated by its goodness of fit to the least-squares regression line, reflects the fact that hindlimb twitching occurred at a constant rate and randomly with respect to time. In contrast, all of the other curves exhibit nearly identical distributions that are composed of two parts as indicated by their fit to the least-squares regression line (for clarity, only the regression lines for the forelimbs and hindlimbs of transected pups are depicted in Figure 3). First, there is an excess of short intertwitch intervals (≤ 3 s) in relation to expected, random frequencies. Second, intertwitch intervals greater than 3 s occurred randomly with respect to time. Finally, forelimb twitching at these longer intervals occurred at the same rate as the hindlimb twitching of transected pups, as indicated by the similar slopes of the two regression lines at these longer intervals.

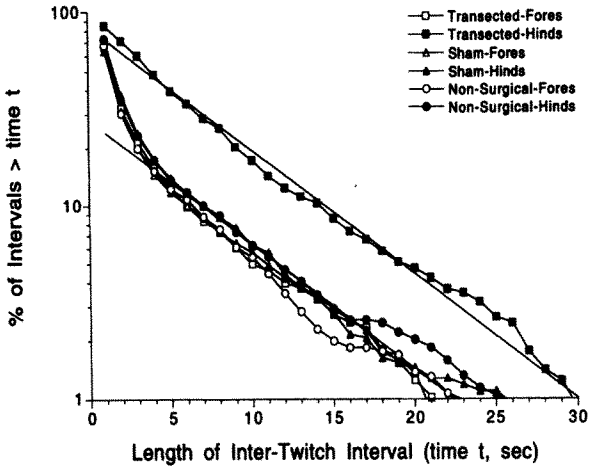

Figure 3. Log-survivor plots of the temporal distribution of twitching in the forelimbs (open symbols) and hindlimbs (solid symbols) of the transected (squares), sham (triangles), and nonsurgical (circles) pups at 5 days of age. Data were pooled within each experimental group. Least-squares regression lines are indicated for the data for the forelimbs and hindlimbs of transected pups.

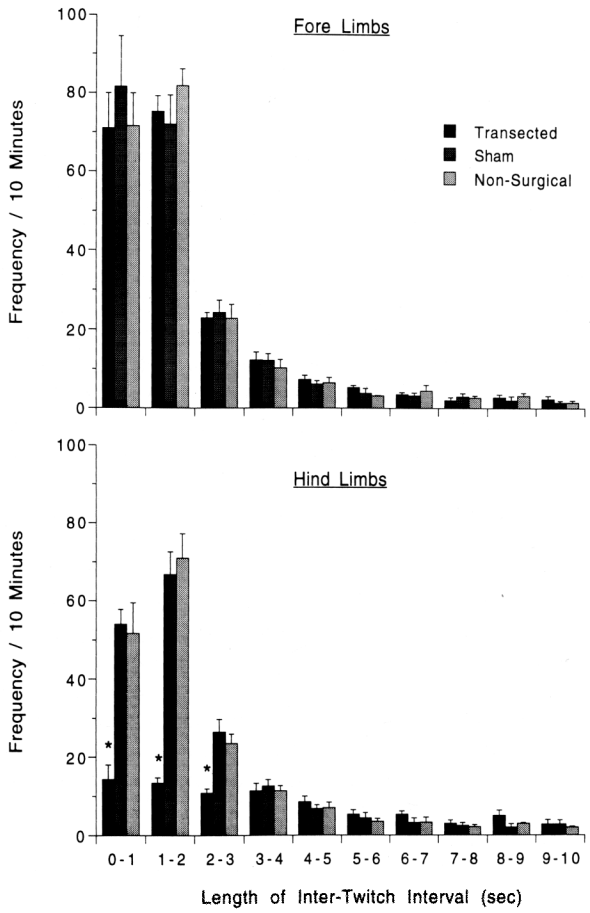

Figure 2. Frequency distributions of intertwitch intervals for forelimbs (top) and hindlimbs (bottom) of the transected, sham, and nonsurgical pups at 5 days of age. $n = 6$ for each group. Mean ± SE_M. *$p < .001$ in relation to sham and nonsurgical controls.

Determination of interlimb synchrony. By disconnecting neural transmission between the motor neurons serving the forelimbs and hindlimbs, it was expected that the transected pups would exhibit less interlimb synchrony than the sham and nonsurgical control pups. To measure relative differences in interlimb synchrony, each 10-min test was divided into $10 \times 60 = 600$ 1-s bins. Then, for each pup, the number of 1-s bins in which both a forelimb and a hindlimb twitch occurred was determined. Next, this number was divided by the number of bins in which either a forelimb or a hindlimb twitch occurred. This proportion is an estimate of interlimb synchrony (see Smotherman & Robinson, 1986).

Figure 4 presents the results of this analysis. First, the synchrony scores for the sham and nonsurgical pups were 46.2 ± 1.5 and 47.1 ± 2.0, respectively. In other words, whenever a twitch occurred within a 1-s bin, there was nearly a 50% chance that a forelimb and hindlimb twitch occurred synchronously (i.e., within that same bin). In contrast, spinal transection reduced this probability to 20.6 ± 1.0. This is significantly different from both the sham and nonsurgical groups, $F(2, 15) = 102.619, p < .0001$.

The synchrony score described above provides a measure of the

probability of synchronous twitching given that a twitch occurred. Thus, it is a useful measure for comparing synchrony scores between experimental groups. But, because one expects a positive synchrony score based on chance associations alone, we need another measure of synchrony in order to determine whether pups were twitching synchronously above levels expected by chance. To do this, we used a simple model of chance associations (Smotherman & Robinson, 1986). First, the probabilities of forelimb twitching and hindlimb twitching within a 1-s bin during the entire 10-min test were determined. Next, these two probabilities were multipled to arrive at the joint probability, a measure of the likelihood that synchronous twitches would occur during a 1-s bin by chance alone. Finally, for each pup, the joint probability was compared with the observed probability of synchronous twitching.

Figure 5 presents the observed and expected frequencies of synchronous twitching. First, it can be seen that the expected frequencies of synchronous twitching were greater in the sham and nonsurgical pups than in the transected pups; this difference is the result of greater rates of twitching in the control pups. Next, it can be seen that, for all three experimental groups, observed frequencies exceeded expected frequencies. For the sham and nonsurgical pups, the differences were 10.0 ± 0.5 and 10.2 ± 0.6, respectively: These differences are highly significant; sham: paired $t(5) = 19.96, p < .0001$; nonsurgical: paired $t(5) = 20.88, p < .0001$. For the transected pups, the difference was only 3.2 ± 0.3; this is also a significant deviation from chance, paired $t(5) = 10.66, p < .0001$.

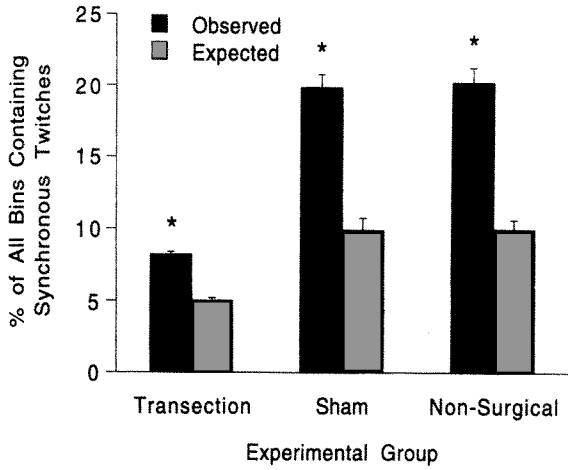

Figure 5. Observed and expected frequencies of synchronous twitching for the transected, sham, and nonsurgical pups at 5 days of age. To derive these values, we determined the probabilities of forelimb twitching and hindlimb twitching within a 1-s bin during the entire 10-min test. Next, these two probabilities were multiplied to arrive at the joint probability, a measure of the likelihood that synchronous twitching would occur during a 1-s bin by chance. Finally, for each pup, the expected joint probability was compared with the observed probability of synchronous twitching. $n = 6$ for each group. Mean ± SE_M. *$p < .0001$ in relation to expected frequency.

Discussion

The present results suggest that there are at least two mechanisms underlying neonatal hindlimb twitching: (a) a random, neurogenic mechanism related to the endogenous bursting of spinal motor neurons (as evidenced by the persistence of hindlimb twitching in the transected pups), and (b) a mechanism that provides descending activation of spinal motor neurons. These findings are consistent with observations in spinally transected rat fetuses in which cyclic body movements are produced by mechanisms located within the caudal spinal cord as well as more rostral structures (Robertson & Smotherman, 1990).

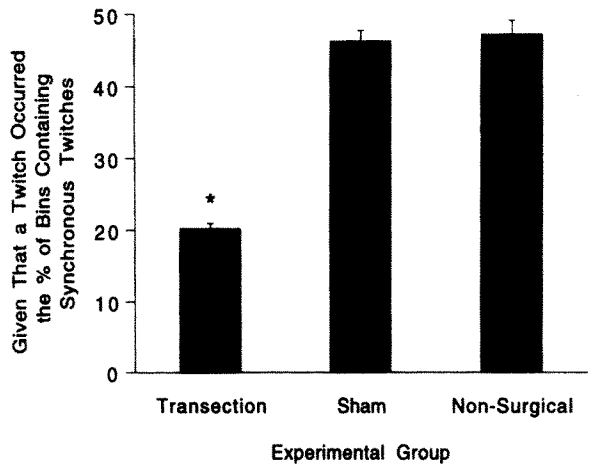

Figure 4. Measures of interlimb synchrony for the transected, sham, and nonsurgical pups at 5 days of age. To derive the measure of interlimb synchrony, we divided each 10-min test into 600 1-s bins. For each pup, the number of 1-s bins in which a forelimb and a hindlimb twitch occurred was determined. Next, to arrive at the synchrony score, we divided this number by the number of bins in which a forelimb or a hindlimb twitch occurred. $n = 6$ for each group. Mean ± SE_M. *$p < .0001$ in relation to sham and nonsurgical controls.

Based on the evidence available, we cannot determine the source of the descending excitation that resulted in short interval twitching in the sham and nonsurgical pups and in the forelimbs of the transected pups. It seems likely, however, that motor neurons within the brainstem (perhaps within the caudal pontine-rostral medullary reticular formation; Vertes, 1984) activated both the forelimbs and hindlimbs simultaneously and independently.

The descending mechanism that produces short interval, rapid limb twitching also produced the relatively high measures of interlimb synchrony seen in the sham and nonsurgical pups (see Figure 4). The transected pups also exhibited, however, small but significant above chance levels of interlimb synchrony (see Figure 5). Although such synchrony in the absence of neural connectivity could result from non-neural cyclic phenomena or mechanical influences, it is also possible that small scoring biases could have produced these results. We have not determined which of these explanations is correct.

The identification of two mechanisms underlying twitching in neonates forms a conceptual bridge between the locally produced "spontaneous movements" described by neuroembryologists and the descending activation described by sleep researchers (Corner, 1977). The neonate allows us to observe the effects of both local and descending mechanisms simultaneously.

Although pups were studied at 5 and 8 days of age, only the data from the 5-day-olds are presented because the results at the two ages were very similar. Specifically, transected 8-day-olds exhibited a 35% reduction in hindlimb twitching that was due entirely to a reduction in short interval twitching. The fact that the results were so similar at both ages adds confidence in the reliability of the findings as well as in the scoring procedures. Systematic developmental studies are necessary to determine the relative contributions of these two mechanisms throughout ontogeny.

These results support and extend Corner's conception of brainstem-activated myoclonic twitching as a "vestige of an originally diffuse endogenously bursting system" (1985, p. 182). Thus, from an evolutionary perspective, spontaneous motility is a universal feature of embryonic and neonatal behavior in vertebrates that, during development in mammals and birds, coalesces with a series of other electroencephalographic, physiological, and electromyographic components to form the seemingly cohesive state now commonly referred to as REM sleep (Corner, 1977).

Hindlimb twitching in newborn rats with spinal transections has been observed by others as well (e.g., Corner, 1973). For example, in one article Corner (1985, p. 181) mentions, in passing, that "neonatal spinal rats exhibit spontaneous twitching which appears to be phenomenologically indistinguishable from the motility" that is used to identify the presence of REM sleep. That myoclonic twitching can, on the one hand, be used as a marker for REM sleep and yet, on the other hand, be retained when neuronal connections between the brain and limbs are severed is inexplicable in terms of any current REM hypothesis.

A review of the literature reveals that the vast majority of studies of REM sleep concern adult mammals; thus, not surprisingly, most REM sleep hypotheses, although relevant to adult functioning (Winson, 1993), are difficult to apply meaningfully to the developing neonate. In 1966, Roffwarg, Muzio, and Dement posited a brain maturation function for REM sleep; this article is still the preeminent citation when reference is made to a function for REM sleep during ontogeny, thus testifying to a relative disinterest in REM sleep as a neonatal phenomenon (Vertes, 1984). But any account of REM sleep must explain its ontogenetic characteristics, unless one proposes that although REM sleep predominates in neonates, it only acquires functional significance as it becomes a relatively rare phenomenon in adults.

What might be the functional significance of twitching? In answering this question with regard to the spontaneous movements of embryos that are generated by local spinal circuits, neuroembryologists have suggested and tested a number of hypotheses. For example, spontaneous embryonic movements appear important for the development of the skeletomuscular system. This hypothesis is supported by the finding that immobilization of the embryo with curare prevents normal growth of muscles and joints (Oppenheim, Pittman, Gray, & Maderdrut, 1978). It has also been hypothesized that embryonic motility regulates the natural occurrence of motor neuron cell death. This hypothesis is supported by the finding that cell death is reduced by blockade of embryonic neuromuscular activity (see Oppenheim, 1989).

Motor neuron cell death is largely completed prenatally. Postnatally, a new competition begins between motor neurons vying for innervation of individual muscle fibers. Specifically, neonatal muscle fibers initially receive multiple inputs from a number of different axons (referred to as polyneuronal innervation; Purves & Lichtman, 1980). These multiple inputs are pruned during development until each muscle fiber is innervated by a single motor neuron.

Twitching could play a role in synapse elimination. Specifically, synapse elimination at each muscle fiber requires that there be "some critical identifying characteristic that distinguishes the members of one synaptic cartel [i.e., group of synapses that share a common motor unit] from members of other cartels innervating the same junction" (Colman & Lichtman, 1993, p. 3). The random, asynchronous firing of individual motor units underlying twitching could provide the necessary unambiguous distinction between synaptic cartels belonging to different motor neurons.

If REM-related twitching is associated with polyneuronal innervation, then both twitching and synapse elimination should exhibit similar developmental trajectories. In fact, this is the case. Specifically, between 10 and 15 days of age in rats, the percentage of soleus muscle fibers that are polyneuronally innervated decreases from 100% to nearly 0% (Brown, Jansen, & van Essen, 1976). Similarly, REM-related twitching in rats drops precipitously between 10 and 15 days of age (Blumberg, 1993; Gramsbergen et al., 1970; Jouvet-Mounier et al., 1970). Interestingly, during this same period, the locomotor abilities of neonatal rats improve dramatically (Altman & Sudarshan, 1975); for example, rats that can only crawl at 8 days of age are walking by 15 days of age.

In addition to possible roles in synapse elimination, spontaneous motor neuron activity may also participate in the establishment of orderly axonal connections between neurons and their target muscle sites (Smith & Hollyday, 1983). Such a role for spontaneous neural activity has been established in the developing visual system (Galli & Maffei, 1988; Meister, Wong, Baylor, & Shatz, 1990; Shatz, 1990; Stryker & Harris, 1986). Specifically, spontaneous activity of retinal ganglion cells contributes to the topographic organization of neural inputs to the lateral geniculate nucleus and primary visual cortex. We are suggesting that spontaneous activity in the spinal cord may play a similar role in the topographic organization of the neuromuscular system.

Thus, spontaneous neural activity, whether in the spinal cord, retina, or elsewhere, may represent a fundamental, universal feature of developmental organization. If so, then REM sleep may be composed of many independent processes, some of which contribute to basic developmental processes, such as synapse elimination and the fine-tuning of topographic relations. Unlike most REM hypotheses, this hypothesis has the distinct advantage of explaining why REM sleep is so much more prevalent in neonates than in adults. Furthermore, we believe such a developmental perspective is essential if we are to begin understanding and integrating those phenomena commonly associated with REM sleep.

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